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THE INFLUENCE OF CERTAIN AGENTS IN DESTROYING THE VITALITY OF THE TYPHOID AND OF THE COLON BACILLUS.

DURING the last year a series of researches upon the influence of light, of desiccation, and of the products of certain micro-organisms upon the vitality of some of the pathogenic bacteria has been carried on in the Laboratory of Hygiene of the University of Pennsylvania, by Dr. Adelaide W. Peckham, in accordance with a general scheme for such investigation prepared by Dr. Weir Mitchell and Dr. Billings, the Director of the Laboratory, and with the aid of a grant from the Bache fund. A portion of the results obtained in this research has been communicated to the National Academy of Sciences at its meetings in April and in October, 1894; but as the volume of the Transactions of the Academy which will contain these papers will not be issued before next year, it has been thought best to publish some account of these experiments without further delay.

That direct sunlight kills or stops the growth of certain bacteria has been known since 1877, when Downes and Blunt presented to the Royal Society a report on "Researches on the effects of light upon bacteria and other organisms."* Since that date a number of papers on this subject have been published, the most important one in relation to the typhoid bacillus being that of Janowski in 1890.† The first series of experiments by Dr. Peckham was made with the *staphylococcus pyogenes aureus*, the object being mainly to determine the best methods of investigation.

* Proc. Roy. Soc. 1877, vol. 26, p. 488.

† Zur Biologie der Typhus Bacillen, Centralbl. Bakteriol, etc., VIII., 1890, pp. 167, 193, 230, 262.

Photobacteriographs were made by Buchner's method, namely, by placing a square of black paper, or of glass of different colors, upon the bottom of a plate containing inoculated agar-agar during insolation; but although the protected portion was visible after fifteen minutes' insolation and incubation for twenty-four hours, and sharply defined after two hours' insolation and incubation as before, no accurate estimate could be made of the difference in the destructive power of different periods of insolation. Successful photobacteriography requires inoculation of large quantities of bacteria, in order that the colonies may be set so closely together that a ground-glass appearance is produced; in which case counting of the colonies is practically impossible.

For this reason the following method was used for each of the three organisms, the *staphylococcus pyogenes aureus*, the *bacillus coli cammunis* and the *bacillus typhi abdominalis*.

To obtain an accurate measure of the effects produced by lights of different intensity or of different colors, it is necessary to ensure, as far as possible, that the bacteria to be experimented on shall be uniformly distributed in the culture media. Tubes containing each 10 cc. of bouillon were inoculated with one drop of a bouillon culture and then placed in an incubator for twenty-four hours. A small quantity of sterilized gravel was then added to the culture tube and it was thoroughly shaken, after which 10 cc. of a one-half per cent. salt solution was added and the culture drawn into a Nuttall's dropping apparatus. From this, one-twentieth of 1 cc. of the bouillon culture was dropped into a tube of melted agar-agar, which was slowly and thoroughly agitated, and the contents were then poured into a Petri dish, carefully levelled on a levelling tripod over ice water. In the first method used the Petri dishes were found to be so uneven on the bottom that the layer of medium under the protective square was often very thick or very thin as compared with that about the circumference of the plate, and, therefore, comparisons made between the centre and the circumference would be in almost every case unreliable. To overcome this difficulty, just one-half of the plate was shaded with black paper or colored glass.

The plates were then exposed to sunlight, bottom upwards, so as to allow the sun to shine as directly as possible on the inoculated agar-agar. At intervals of fifteen minutes a plate was removed and placed in the incubator. The temperature of the plates during insolation was always below 34° C. as shown by a thermometer with a blackened bulb which was placed in the sun and the temperature noted every fifteen minutes. Sunny, still days were utilized for insolation, beginning at 10 A. M. during the months of October, November and December. After insolation, the plates, and also a non-insolated control plate were incubated for twenty-four hours.

The colonies were counted in the following manner: A number 1 eye-piece was divided into fields (as done by Nuttall in counting tubercle bacilli), by introducing a disk of black cardboard which had a square opening divided into four parts by two hairs placed at right angles. This eye-piece and an objective of low power were used in counting. The percentage of germs destroyed by insolation was estimated from the mean of four counts taken on both the insolated and the protected halves of the plate. By this method an accurate statement can be made regarding the difference in protective power given by the different colors, not from simple observation, but by comparison of a definite number of colonies counted.

The following table shows the comparative effect of the blue rays and of complete

shadows on the growth of the organisms experimented on :

			Percentages of organism destroyed in the insolated half of the plate as compared with the protected half.							
Number of minutes exposed.....			15	30	45	60	75	90	105	120
Typhoid	Shaded with	black paper..	17	28	33	34	65	63	90	98
		blue glass....	7	14	30	32	24	38	35	52
Colon ...	Shaded with	black paper..	25	15	25	71	83	88	97	99
		blue glass....	18	29	32	35	56	59	60	52
Aureus..	Shaded with	black paper..	..	..	55	..	72	72	80	90
		blue glass....	..	38	34	54	51	41	48	50

From this series of experiments the following results were obtained :

Insolation for fifteen minutes destroys to a slight extent each of the three organisms experimented upon. Two hours' insolation destroys 98% of the germs and from three to six hours kills all. The colon bacillus is more easily destroyed by insolation than is the typhoid bacillus. Exposure to diffuse daylight, to gas light, or to the incandescent electric light produces little effect. Red, orange, yellow, and green light produce little effect, during two hours' insolation; while the blue and violet rays kill nearly as rapidly and as certainly as full sunlight. Insolation from six to eight hours lessens the number of colonies under the protective square to a slight extent, for the colors red, orange, yellow and green.

Plates were made in the same manner and exposed to diffused light for periods varying from fifteen minutes to two days. The exposure was made on clear sunny days in the light part of a room. In this experiment the result was negative, the number of colonies on the two sides of the plate being approximately the same.

An ordinary gas-burner and an incandescent light were each used as the source of illumination. The plates were placed bottom-upwards in a dark room near the light used. Illumination for sixteen hours with gas produced no effect on the growth of the organism as shown by counting of the colonies.

Illumination for four and one-half hours with an incandescent light also gave negative results.

A series of experiments was made with tubes of bouillon inoculated with the different organisms and then enclosed in larger tubes containing fluids of different colors—red, orange, yellow and blue, which were exposed to sunlight with control tubes, one placed in water, and the other in a similar tube covered with black paper. The materials used for making the colored solutions were corallin, chromate and bichromate of potassium, and methylene blue. From these tubes, plates were made, and the number of colonies counted.

It was found that an increase in the number of colonies continued to the eighteenth day, the number being greater in the colon and aureus cultures than in the typhoid. The colonies then began to decrease, and on the fifty-eighth day the plates contained but few colonies. In this experiment, as in the last, plates made from culture tubes placed in blue fluid showed fewer colonies.

Since the presentation of the above results, with details, charts and tables, to the National Academy, in April, 1894, Dr. Dieudonné has published in the *Arbeiten aus dem Kaiserlichen Gesundheitsamte*, a paper on the effects of sunlight on bacteria, in which he reports results substantially the same, and obtained by almost the same methods as those of Dr. Peckham.

Sunlight not only weakens or kills the typhoid and the colon bacillus, but it affects culture media so as to render them less capable of supporting the growth of these organisms. Dr. Peckham found that sterile bouillon insolated from one to ten days and then inoculated with the *bacillus typhi abdominalis* showed no diminution in the number of colonies as compared with a control

plate made from a similar culture not so exposed. Twenty days insolation and then inoculation with the typhoid bacillus showed great decrease in the number of colonies on all the plates; some of them were sterile. Insolation of forty days, and inoculation in the same manner, gave very few colonies for each plate, probably the same as the number of germs introduced, *i. e.*, there had been no development. Bouillon insolated 50—60 days and inoculated gave sterile tubes. This insolated bouillon after inoculation and incubation remained perfectly clear, and plates made after a week of incubation gave no more colonies than those made at the end of twenty-four hours. Its reaction was alkaline, but not intensely so.

Insolated agar-agar—Of twenty-three tubes of agar-agar insolated twenty days, and then inoculated with the *bacillus typhi abdominalis*, all except one remained sterile, and neither the *bacillus typhi abdominalis* nor the *bacillus coli communis* grew when inoculated in stripes on these plates. Of seven tubes of agar-agar insolated forty days and then inoculated with the bacillus of typhoid, all remained sterile. On four of these plates mould appeared after some days. Of seven tubes of agar-agar insolated forty days and then inoculated and incubated as before, all remained sterile.

Insolated gelatine.—Of ten gelatine tubes insolated forty days and then inoculated with the *bacillus typhi abdominalis*, six remained sterile, two contained a few colonies of *bacillus typhi abdominalis*, and two were contaminated.

The insolated bouillon was then kept in diffuse daylight for forty days and again inoculated with the typhoid bacillus. Within twenty-four hours the tubes of bouillon betame turbid and plates made from them showed innumerable colonies.

It is difficult to account for the effect of insolation on culture media. Roux in his experiments on anthrax found that insolation of bouillon for *two* or *three hours* rendered it unsuitable for germination of the spores, but if the bacilli were introduced they would thrive. He attributes this alteration to some chemical change which the culture media undergo during the insolation. He found also that if the insolated media were kept in the dark or in diffuse daylight for a time, the original nutritive qualities were restored and germination of spores would take place. Geisler and Janowski observed the bactericidal properties of insolated media, but the latter could find no chemical alteration in such media.

Percy Frankland in his chapter on action of light on micro-organisms* concludes from the results obtained by many investigators 'that the effect is due to a process of oxidation possibly brought about through the agency of ozone or peroxide of hydrogen, or both; that all apparently direct low temperature oxidations require the presence of water. And inasmuch as the bactericidal action of light is unquestionably a case of low temperature oxidation, there is the strongest presumptive evidence, as well as weighty experimental evidence, that moisture, which practically means the possibility of the presence of peroxide of hydrogen or of some similar material, is essential for its manifestation.'† Westbrook ('Some of the effects of sunlight on tetanus cultures, Jour. of Pathol. & Bacteriol. III., Nov. 1894, 71') found that old broth cultures of the tetanus bacillus in an atmosphere of hydrogen were not in the least affected by exposure to sunlight, either in regard to their virulence or their rapidity of growth on reinoculation. When the same culture was sealed up in the presence of air, the

*Micro-organisms in water, p. 390.

†Gelatine, to which were added different amounts of the peroxide of hydrogen, was inoculated with the *bacillus typhi abdominalis* and poured into plates. Those plates in which more than one part of the peroxide to 5000 of gelatine was used, were sterile.

micro-organisms, were not only killed, but the material was completely harmless when inoculated into white mice. It was, however, possible to obtain vigorous and virulent growths from cultures which had been made quite innocuous by the action of the sun. Oxygen was used up in the process. Under ordinary circumstances one might be tempted to explain the effect of sunlight in destroying bacteria by the drying of the organisms exposed to it, especially in the case of those bacteria which do not form spores, but our experiments show that desiccation for months has little effect on the vitality of the typhoid or of the colon bacillus. To determine the influence of desiccation upon these organisms, and also upon the *staphylococcus aureus*, the following experiments were made:

Bouillon cultures of the *bacillus typhi abdominalis*, the *bacillus coli communis* and the *staphylococcus aureus* were roughly dried on threads one centimetre long and then desiccated, a portion being placed in a vacuum, another portion in a desiccator over sulphuric acid, and a third in a closet; all were kept in the dark. The result of the desiccation under the three different conditions is as follows:

Bacillus typhi abdominalis:

Lived in a vacuum from December 30 until July 24, or 207 days. In a desiccator over sulphuric acid from January 3 until July 24, or 213 days.

In a closet from December 18 until July 24, or 229 days.

Bacillus coli communis:

Lived in vacuum from November 29 to May 30, or 183 days.

In a desiccator over sulphuric acid from January 3 until July 24, or 213 days.

In a closet from December 30 until May 30, or 152 days.

Staphylococcus aureus:

Lived in vacuum from November 29 until July 24, or 207 days.

In a desiccator over sulphuric acid from October 25 until April 19, or 178 days.

In a closet from February 13 until July 24, or 162 days.

It will be seen from these experiments that the organisms experimented on endure desiccation for five months, or more, without losing their vitality, and hence the slight evaporation which may have occurred in the insolation experiments, had probably no influence on the results.

It is evident that sunshine must exercise considerable influence in destroying bacteria on the surface of soil, streets, etc., exposed to its influence, but its action is almost confined to the surface, as appears from the results obtained by Esmarch in attempts to disinfect bedding and clothing by this agency. While the light from an incandescent electric lamp has little germicidal effect, that from a powerful arc lamp produces effects similar to those of sunlight, and it has been proposed to use this means to disinfect the walls of infected rooms. The bacillus of tuberculosis appears to be more quickly destroyed by light than the typhoid or the colon bacillus, being killed by exposure to simple diffused daylight in about a week,* and this fact should be borne in mind in advising measures to prevent the diffusion of this organism.

The investigations upon the typhoid and the colon bacillus referred to in this paper, were undertaken as part of a general scheme of inquiry to ascertain the agencies which tend to detroy the typhoid bacillus when it is introduced into a source of water supply, as, for example, into a running stream. An important part of this investigation relates to the influence of the common water bacteria, or of their products, upon the vitality of the typhoid bacillus.

This research was conducted as follows:

* Ueber bacteriologische Forschung: Vortrag in der ersten allgem. Sitzung des X internationalen Congress, 1890.

1. Forty-five varieties of bacteria found in the water of the Schuylkill river were used in the first experiment. Cultures of each organism were made on agar-agar and after attaining a luxuriant growth were sterilized, the reaction was taken, and the medium was again slanted. A set of these tubes was inoculated with the *bacillus typhi abdominalis* and a second set with *bacillus coli communis*.

The object of this research was to ascertain whether the two organisms would grow on media containing the products of the activity of water bacteria. The reaction was alkaline in every tube. The *bacillus typhi abdominalis* and the *bacillus coli communis* lived in every instance, some showing fairly luxuriant growths, while others were only transparent films.

2. In the second experiment, thirty-nine varieties of the water bacteria used in the first experiment were inoculated into tubes each containing 10 cc. of sterilized tap-water and 5 drops of bouillon. Two sets of tubes were made as before, one being inoculated with the *bacillus typhi abdominalis* and the other with the *bacillus coli communis*. To ascertain whether the two organisms under consideration would multiply in the presence of water bacteria, gelatine plates were made for twelve or more days. Both bacilli gave characteristic colonies with each of the water organisms, except two which had apparently an antagonistic effect upon their development. They were both members of the *subtilis* group. In other members of this group this peculiarity was absent.

The typhoid bacillus in several instances outlived its associate organism. In one instance a gelatine plate made from a tube of sterilized water inoculated with the typhoid bacillus and a water bacterium 160 days previously gave characteristic colonies of the *bacillus typhi abdominalis*.

3. To meet the objection that might be raised to the use of heat for the sterilization of the medium in which the water organisms had grown, the opinion having been advanced that some products of growth are either volatile or rendered inert by high temperatures, flasks each containing 70 cc. of bouillon were inoculated with water bacteria and incubated for from 15 to 20 days. The cultures were then filtered through porcelain, the reaction was taken, and the filtrate was run into sterilized tubes which were inoculated with the *bacillus typhi abdominalis* and the *bacillus coli communis* and then incubated. In each of the thirteen filtrates inoculated the bacilli grew and multiplied for at least four days.

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CURRENT NOTES ON PHYSIOGRAPHY (I.).

INTRODUCTORY NOTE.

It is proposed to contribute to Science under the above title a series of notes and comments on recent investigations and current literature concerning physiography, or physical geography in its modern form. A brief statement of the field to be covered may be appropriate at the outset.

Following the plan introduced by Carl Ritter, and popularized in this country chiefly by Arnold Guyot, geography may be defined as the study of the earth in its relation to man. Some prefer to extend this relation to all forms of life. Physical geography may then be defined as the rational study of those features of the earth which must be understood in order to appreciate its relation to man. In deference to the opinions of the majority of the conference on geography, held in Chicago in Christmas week, 1892, physiography is taken as the name of this subject in its modern form, with particular reference to the rational study of the lands, where man dwells. Descriptive geography is an empirical study